Eur Spine J (2000) 9 : 30–35

ORIGINAL ARTICLE

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Tissue response to bioactive glass and autogenous bone in the rabbit spine

Received: 6 May 1999
Revised: 17 September 1999
Accepted: 28 October 1999

This work was financially supported by the Finnish Orthopedic Association and the Medical Society of Finland.

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Abstract Bioactive glass S53P4 and autogenous bone were used as bone graft materials in an experimental rabbit model for spinal fusion. The study focused on differences in bone formation using bioactive glass and autogenous bone as bone graft materials. Bioactive glass, a mixture of bioactive glass and autogenous bone or autogenous bone was implanted for 4 and 12 weeks at the thoracolumbar level. Undecalcified sections were prepared for histological and histomorphometric evaluation. New bone formation was seen in all implanted areas, with the bone growing from the surface of the vertebrae enclosing both glass and autogenous bone in the bone fusion mass. During the observation period, the measured amount of bone remained at the same level in the autograft group, while in the glass and the glass/autograft bone groups it increased. By 12 weeks, no significant difference in bone formation between the three groups was observable. The bone formation in two selected standardized areas at 12 weeks was 21 and 24% in the glass group, 23 and 28% in the glass/autograft bone group and 27 and 26% in the autograft bone group. We consider bioactive glass as a potential bone graft material in experimental spinal fusion.

Key words Bioactive glass · Spinal fusion · Autogenous bone · Histomorphometry

Introduction

Posterior spinal fusion was first performed by Russell Hibbs in 1911. In the operation, spinal fusion was obtained by decorticating the spinous processes, laminae and facet joints and by adding autogenous bone from the iliac crest. Posterior spinal fusion is still a combined common procedure for spinal stabilization. The operation requires large amounts of bone graft material, which is not always available. Bone graft substitutes are, therefore, of great interest.

Trials to achieve spinal fusion in animal models have been made with several bone graft materials, such as natural coralline calcium carbonate, hydroxyapatite, tricalcium phosphate, Bioglass, macroporous biphasic calcium phosphate, decalcified bone matrix, deep-frozen allogenic bone and autograft bone [5, 6, 8, 10, 11, 14, 17–19, 21]. Trials with several bone matrix proteins have also been reported [3, 4]. Few studies include histomorphometric data. Depending on the experimental model, bone graft material and evaluation model, new bone formation of very varying degrees has been reported.

Bioactive glasses are surface-active bone graft materials, which have shown both good biocompatibility and good osteoconduction. The reactivity of the glass is based on the formation of a hydroxycarbonate apatite layer on the glass surface, to which bone can chemically bind [1–9, 12–16, 20].

In this study, a bioactive glass, S53P4 [2], was studied as a possible bone graft substitute for use in experimental spinal fusion. The aim of the study was to answer several questions. Does bioactive glass incorporate into the bone fusion mass? Do any adverse tissue reactions occur? Are

there significant differences in bone formation when bioactive glass and autogenous bone are compared? As the healing process and the biology in spinal fusion are not fully understood, this study focused on histomorphometric and histological examinations.

Materials and methods

Glass preparation

The bioactive glass, S53P4, composed of 53% SiO_2, 23% Na_2O, 20% CaO and 4% P_2O_5, was chosen for the experiment [2]. The raw materials used were SiO_2, Na_2CO_3, $CaCO_3$ and $CaHPO_4.2H_2O$. The glass was melted in a platinum crucible for 3 h at 1360 °C. After casting in a preheated graphite mold, it was crushed and remelted for homogenization. The glass was crushed again and sieved to a fraction of 630–800 μm.

Animals and surgery

Sixteen 6-month-old adult rabbits, whose weight ranged from 2.9 to 5.3 kg (average 4.1 kg) were used. The operations were performed with the animals under general anesthesia, using ketamine hydrochloride, medetomidine hydrochloride and diazepam.

A dorsal incision was performed at the thoracolumbar level of the back. The line of processus spinosus was identified and exposed by moving the muscle-fasciae tissue laterally. The lamina and the region of the articular processes were carefully exposed. Bone graft material was placed posteriorly in the exposed regions in two adjacent thoracal vertebrae. The bone graft material was (1) glass, (2) glass-autograft bone mixture (70/30 vol%) and (3) autograft bone. Each bone graft material was implanted eight times in a randomized manner. The autograft bone was taken from the iliac crest by a second operation. The wounds were closed in layers.

Eight vertebrae served as a control group. The region of the vertebrae was exposed, but no graft material was implanted.

After the operation, all animals received a single subcutaneous prophylactic dose of benzylpenicillin and the analgesic buprenorphine hydrochloride for 3 days.

Eight animals were killed at 4 weeks and eight animals at 12 weeks.

Preparation of specimen

The vertebral columns containing the implants were explanted and fixed in buffered formaldehyde. The vertebral column was sectioned into two halves along the mid-axis in sagittal planes. The samples were dehydrated in increasing concentrations of ethanol and methylmethacrylate for 2 months, and finally embedded in methylmethacrylate (Technovit, Kulzer GmbH, Wehrheim, Germany). Using a cutting-and-grinding technique [7], 20-μm thick undecalcified sections were prepared. The sections were stained with toluidine blue, and the van Gieson and Kossa method.

Preparation of the glass and the specimens was performed according to the standards of good laboratory practice.

Histomorphometry

The histomorphometric measurements were performed using MicroScaleTC (Digithurst Ltd., UK), a PC-based color image analysis system.

The amount of bone and glass granules located in the sections was measured in two standardized areas:

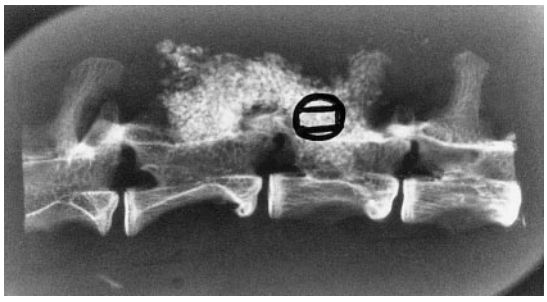

Fig. 1 Radiograph of sample embedded in methylmethacrylate. The area for histomorphometric measurements is visualized

1. A rectangle with sides of 3 and 5 mm, and
2. A circle with a radius r = 2.5 mm

The position of the rectangle and the circle is shown in Fig. 1. The histomorphometric measurements were performed using the specimens stained with the van Gieson method, as it was apparent that this method achieved the best contrast between bone and soft tissue [1].

Results

All histological sections showed new bone formation in the implanted areas without any cartilage formation or any adverse round cell tissue reactions. At 4 weeks, this intramembranous bone consisted partly of woven bone and partly of trabecular bone. Later, at 12 weeks, it consisted of trabecular bone resembling the bone structure found in the intact vertebrae. The bone was growing from the surface of the vertebrae, enclosing both glass granules and autograft bone in its network (Fig. 2). The bone mass formed was located at a distance of 5–7 mm above the corpus arcus of the vertebrae. Glass and autograft material was also located more dorsally, but at this level, at the periphery of the grafted area, no new bone formation or connective tissue was observable. New bone was also directly attached to the surface of the glass granules, which had developed visible reaction zones (Fig. 3). In the control group, in which no graft was implanted, no new bone formation was evident.

Circle

At 4 weeks, the average bone formation in the glass group was 13%, in the glass/autograft group 20% and in the autograft bone group 26%. At 12 weeks, bone formation had increased in the glass group and to a lesser extent in the glass/autograft group, but in the autograft bone group no difference in new bone formation was observable (Fig. 4 A, Table 1). The measured amount of bone in the glass group was 21%, in the glass/autograft group 23% and in the autograft bone group 27%. The average per-

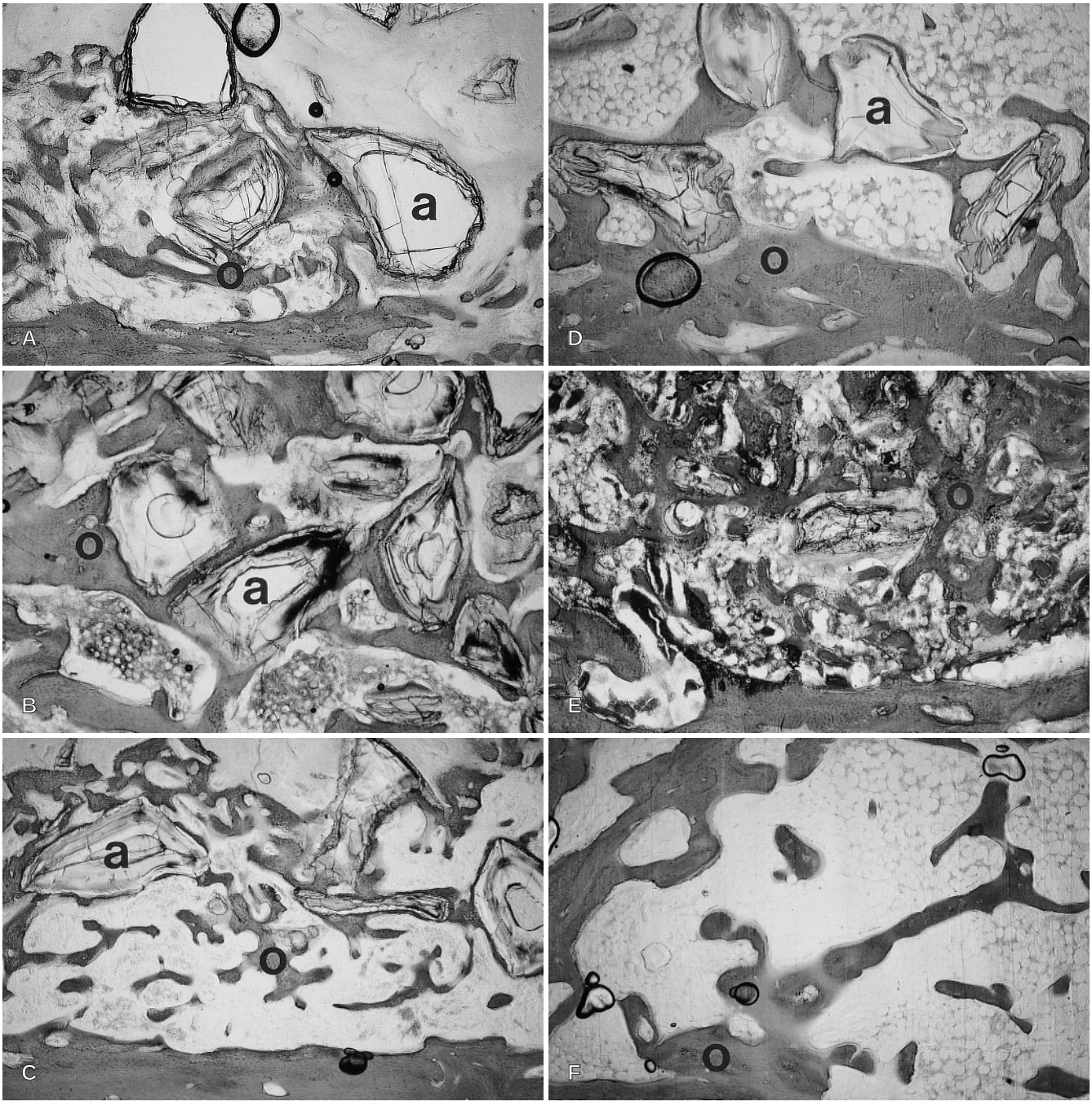

Fig. 2 A–F S53P4 glass and autogenous bone implant at the thoraco-lumbar level in rabbit. Implanted material/implantation time in weeks: **A** glass/4, **B** glass/12, **C** glass-autogenous bone/4, **D** glass-autogenous bone/12, **E** autogenous bone/4, **F** autogenous bone/12. (*o* bone, *a* glass, ×40)

centage of glass at 4 weeks and 12 weeks was 26 and 23% in the glass group and in the glass/autograft group 22 and 16%.

Rectangle

At 4 weeks, the average bone formation in the glass group was 19%, in the glass/autograft group 22% and in the autograft bone group 25%. At 12 weeks the formation of bone had increased both in the glass group and the glass/autograft bone group (Fig. 4 B, Table 1). No difference in bone formation was observable for the autograft bone group. The formation of bone in the glass group was 24%, in the glass/autograft bone group 28% and in the autograft bone group 26%. The average percentage of glass

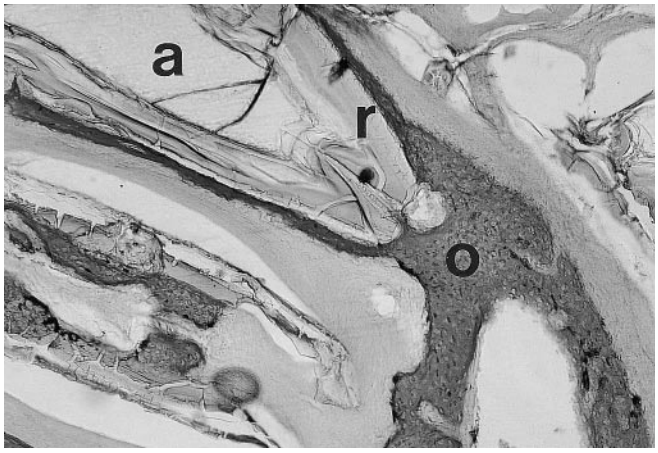

Fig. 3 New bone attached to the surface of a glass granule. (*R* reaction zone, *o* bone, *a* glass, ×100)

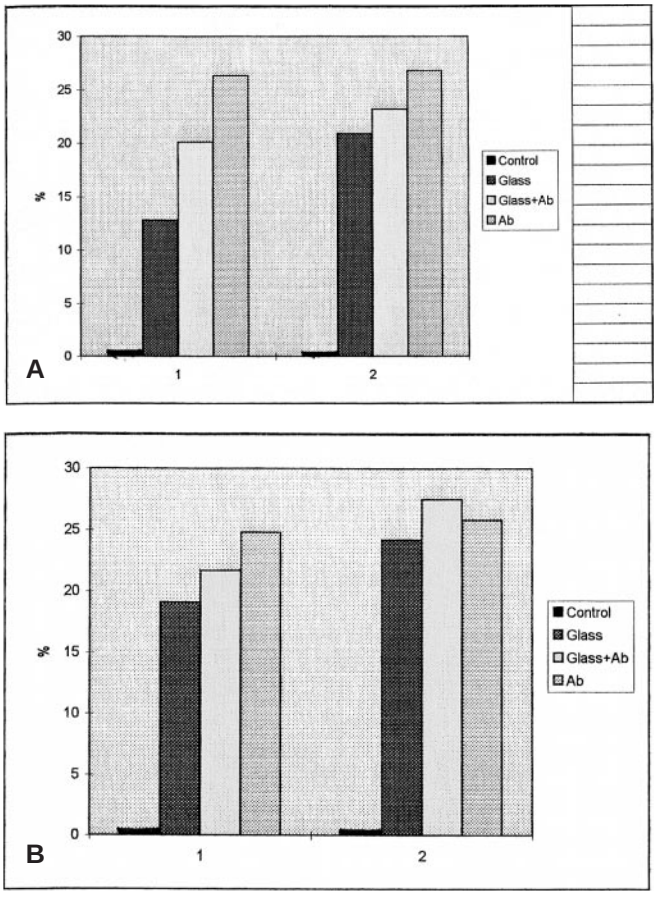

Fig. 4 **A** Measured amount of bone (%) in the glass, glass/autogenous bone and autogenous bone groups in the circle: 1 = 4 weeks, 2 = 12 weeks. **B** Measured amount of bone (%) in the glass, glass-autogenous bone and autogenous bone groups in the rectangle: 1 = 4 weeks, 2 = 12 weeks

in the glass group at 4 and 12 weeks was 29 and 17% and in the glass/autograft group 18 and 12% respectively.

The remaining 52–75% of the observation area consisted mainly of loose fibrillar connective tissue with collagen fibers of varying thickness, corresponding to normal intertrabeculous tissue seen in the vertebral anatomy. Some empty areas due to technical reasons and resorption, but without quantitative correlation to graft material, were also observable.

Discussion

Our study shows that the bioactive glass S53P4 has potential as bone graft material in the rabbit spine. There are no significant differences between the bioactive glass groups and the traditional autograft groups at 12 weeks regarding new bone formation in spinal fusion. The bioactivity, biocompatibility and osteoconductivity of the glass results in a glass-bony network, in which the glass is incorporated into the bone fusion mass. We did not observe any adverse tissue or inflammatory cell reactions in any of the three groups.

The suitability of bioactive glass material as a bone substitute for clinical spinal fusion has, however, to be considered carefully, as bone healing in a rabbit model is known to have a high bone formation rate. In spite of this limitation, our study gives support to clinical use of bioactive glass in the spinal region.

We chose the 70/30 ratio for the mixture of glass and autogenous bone, to see how a small amount of autograft bone would affect the bone formation, and whether the results would be nearer the results for the glass or the autograft bone group. Clinically, there is generally not much autograft bone available. We were therefore interested to see whether a small amount of autograft bone would be sufficient for bone formation.

To express bone formation in an objective and standardized way presented some difficulties. The cutting line of the specimens varied to some degree, because of technical and anatomical reasons. Thus, several sections were made to find a standard area for comparable histological measurements. We finally measured the amount of bone and glass in two selected areas. The first area, the circle, describes the bone formation more distant from the vertebrae and gives an “overview”, a mean value of the bone formation in the selected area. The second area, the rectangle, describes the bone formation near the vertebrae. We observed that this is the place where bone formation starts and where bone formation after a short time interval can best be compared. The measured amount of bone in the area of the circle (i.e., overview) is, therefore, generally smaller than that in the rectangle (i.e., near the vertebrae), which is explained by the fact that the circle includes areas in which no bone formation has yet occurred.

During the observation period, the amount of bone in the glass and glass-autograft groups increased. However,

Table 1 Histomorphometric measurements of bone (%) and glass (%) in circle and rectangle (*Glass + Ab* = 70% glass and 30% autogenous bone, *Ab* autogenous bone)

Area/graft material	Weeks	Bone		Glass	
		Mean	SD	Mean	SD
Circle					
Glass	4	12.8	4.1	26.1	9.3
Glass + Ab	4	20.1	10.3	21.5	8.0
Ab	4	26.3	9.3	–	–
Glass	12	20.9	5.9	23.2	5.2
Glass + Ab	12	23.2	9.7	15.6	8.0
Ab	12	27.0	8.6	–	–
Rectangle					
Glass	4	19.1	3.9	18.9	9.2
Glass + Ab	4	21.7	7.6	17.7	6.6
Ab	4	24.8	11.6	–	–
Glass	12	24.2	8.5	16.8	6.6
Glass + Ab	12	27.5	6.2	11.6	5.0
Ab	12	25.8	5.8	–	–

in the autograft group the amount of bone at 4 and at 12 weeks is almost the same. We believe that this is explained by the high resorption rate of autograft bone in the beginning of the healing process [17]. During the observation period, the amount of glass decreased. This is due to the durability of the glass, as it is believed that the glass will undergo reactions, dissolve and be replaced by bone in time. The remainder of the morphometric area (52–75%), which is characterized predominantly by fibrous tissue and some empty areas, is, because of the resorption process, quite high, especially for autogenous bone. Bone, with the exception of cortical bone, is not throughout a solid tissue. Besides trabeculae, it consists of numerous cavities of intertrabecular tissue, which, in histological sections, consist of hematopoietic or fibrous tissue or appear empty. These empty cavities in the bone mass are not included in the measurements of bone, which will affect the results, especially in the autogenous bone group. However, the measured amount of solid bone, which provides the frame in the fusion process, is comparable across the three groups.

In a posterolateral lumbar intertransverse process arthrodesis in rabbit, Boden et al., describing a healing sequence of 10 weeks, reported a total bone area of less than 30% for autogenous bone graft at the end of the 10 weeks [3]. This is in accordance with our observations for autogenous bone. During the late observation period they also reported a decrease in total amount of bone. Similar results with partial resorption of the graft material have been reported by others [6].

Most authors describe the histology without quantitative analyses. To our knowledge, the only bioactive glass used in experimental spinal fusion is Bioglass (BG). To determine the potential for augmenting and enhancing spinal fusion, Nasca et al. implanted BG in dogs. Histologic evaluations showed a thin, fibrous encapsulation with some adjacent bony trabeculae [17]. This is in contradiction to our findings, that granules of bioactive glass S53P4 show good structural contact with bone. The corresponding experiment for hydroxyapatite (HAP) and tricalcium phosphate (TCP) revealed new bone formation surrounding and incorporating the HAP particulate and bony trabeculae near the TCP particulate [18]. Cook et al. found extensive fibrous tissue infiltration around HAP implants. The fibrous tissue was, however, replaced in 12–24 weeks by new bone [5].

Conflicting results have also been reported for the type of bone formation process. Some authors suggest that endochondral bone formation is involved in the healing process, while others believe it to be primary intramembranous bone formation [1, 3, 14, 21]. Boden et al. have shown that both primary membranous and endochondral bone formation is involved in the healing process of spinal fusion [3]. In our study, no endochondral bone formation was observable in any of the three groups.

It is apparent that by producing local surgical trauma on the vertebrae, the periosteal cells can be activated. The bioactive glass then serves as an osteogenic stimulus for bone formation and the bone will grow from the vertebrae between the granules, resulting in glass-bone bonding. The same phenomenon has been found using bioactive glass as filler material in experimental and clinical drilled holes in bone [12, 16]. This study, therefore, enhances the understanding of bone formation using bioactive glass as bone graft material, and indicates that bioactive glass has potential as bone graft material in the rabbit spine. This will encourage us to further investigations focusing particularly on the emergence of fusion.

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